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## Description

This invention relates to starch hydrolysing enzymes. More specifically, the present invention is directed to chimeric alpha-amylases, to processes for preparing such chimeric alpha-amylases and to the use thereof for the overall enzymatic conversion of starch into high DX syrups, the term DX meaning percentage by weight of dextrose (D-glucose) calculated on the basis of dry substance (DS) of the syrup.

## 2. BACKGROUND OF THE INVENTION

The overall enzymatic process generally adopted by manufacturers of high DX syrups from starch entails two-stages: liquefaction and saccharification. The first step, the liquefaction, involves the hydrolysis of starch into a mixture of oligosaccharides, the so called maltodextrins. This process is catalyzed by alpha-amylases at a temperature of at least 75 °C, preferably at about 90 °C or by a jet-cooking process wherein the starch slurry is heated for at least several minutes to 105-110 °C, usually with a single dose of alpha-amylase, and then held at about 90 °C for at least one hour.

A variety of microbial, particularly bacterial, alpha-amylases are commercially available for the liquefaction process, for example BAN<sup>TM</sup> (from *Bacillus amyloliquefaciens* and TERMAMYL<sup>®</sup> (from *Bacillus licheniformis*), both supplied by NOVO INDUSTRI A/S, Denmark, and THERMOLASE<sup>TM</sup> (from *Bacillus stearothermophilus*) available from Enzyme Development Corporation, N.Y., U.S.A. While BAN alpha-amylase is only stable up to about 85 °C and hence barely suitable for the jet-cooking process, both the TERMAMYL and THERMOLASE enzymes are well adapted for this almost globally preferred mode of starch liquefaction because they are heat stable.

The saccharification step, in which the maltodextrins are converted into dextrose, is mostly catalyzed by a glucoamylase enzyme. Commercial glucoamylase preparations, usually derived from *Aspergillus* or *Rhizopus* species, are available from various manufacturers, e.g. as AMG<sup>TM</sup> 200L, a product obtained from *Aspergillus niger* and manufactured by NOVO INDUSTRI A/S, Denmark.

With a view to further increasing the dextrose yield from 30 - 40 percent by weight DS maltodextrin solutions it has become customary to conduct the saccharification process with glucoamylase in the presence of a debranching enzyme in order to facilitate the hydrolysis of branched oligosaccharides originating from the amylopectin portion of starch. One such debranching enzyme with maximum activity in the same pH and temperature ranges as glucoamylase is disclosed in European Patent Application No. 82302001.1 (Publication No. 0063909). The debranching enzyme is marketed by NOVO INDUSTRI A/S, Denmark, either as such under the proprietary name, PROMOZYME, or as a composition with suitable admixture of glucoamylase under the proprietary name DEXTROZYME.

Unfortunately, the otherwise very favorable combination of *B. licheniformis* alpha-amylase for liquefaction and glucoamylase-PROMOZYME for saccharification in the conversion of starch to high DX syrups entails an inconvenience. It has been observed that the presence of residual alpha-amylase activity from the liquefaction stage has a negative effect on the maximum DX obtainable by saccharification with glucoamylase-PROMOZYME. The problem is greatest with the thermostable *B. licheniformis* alpha-amylase which is still active at the preferred conditions for saccharification (of about pH 4.6 and temperature of about 60 °C, respectively). A remedy has been devised consisting of inactivation of the alpha-amylase prior to saccharification by acidification of the liquefied starch to a pH below 4.5 while maintaining a temperature of at least 90 °C. Following inactivation of the alpha-amylase, the temperature and pH are adjusted to saccharification conditions, meaning that the pH has to be brought up to about 4.5. This additional pH adjustment inevitably increases the salt content of the syrup and hence the expenses connected with desalting the final syrup.

The object of the present invention is to overcome the above-mentioned inconveniences still associated with the use of *B. licheniformis* alpha-amylase for the conversion of starch into a high DX syrup. This and other objects which will be dealt with subsequently in this specification are attained by conducting the liquefaction process with a novel type of alpha-amylase.

## 3. SUMMARY OF THE INVENTION

The chimeric alpha-amylase enzymes of the invention comprise all or portions of the amino terminus of the alpha-amylase derived from *B. amyloliquefaciens* joined to the carboxy terminus of the alpha-amylase derived from *B. licheniformis*. Briefly stated, the present invention provides chimeric alpha-amylases, which are thermostable and exhibit a reduced negative effect on the use of *A. niger* glucoamylase and *B. acidopullulyticus* pullulanase for the saccharification of starch, having the general formula I

## (I)

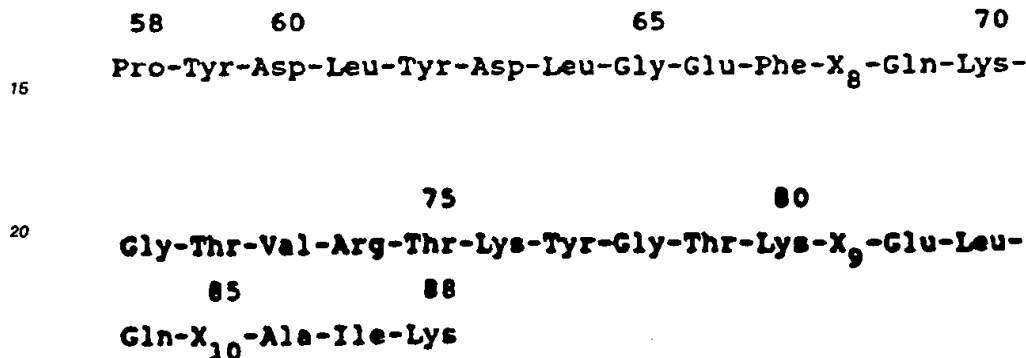
## Q-R-L

5 in which Q is a N-terminal part of from 55 to 60 amino acid residues which is at least 75 percent, preferably at least 80 percent, and more preferably at least 90 percent homologous to the 57 N-terminal amino acid residues in the Bacillus amyloliquefaciens alpha-amylase (Takkinen, et al., 1983, J.Biol.Chem. 258:1007-1013);

R is a part of the general formula Ia:

10

## (Ia)



in which

- 30 X<sub>8</sub> is His or Gln,  
 X<sub>9</sub> is Gly or Ser,  
 X<sub>10</sub> is Ser or Asp; and

L is a C-terminal part of from 390 to 400 amino acid residues which is at least 75 percent, preferably at least 80 percent, and more preferably at least 90 percent homologous to the 395 C-terminal amino acid residues in the Bacillus licheniformis 584 (ATCC 27811) alpha-amylase (Stephens et al., 1984, J.Bacteriol. 158:369-372).

35 Because of the relevance of Takkinen et al., *supra*, and Stephens et al., *supra*, in defining the amino acid sequences of the alpha amylases produced by B. amyloliquefaciens and B. licheniformis, portions of which sequences are contained within the chimeric amylases of the invention, these references are incorporated by reference herein in their entirety.

40 The amino acid sequence of the chimeric enzymes described and shown above may be modified by the substitution, deletion or addition of amino acid residues within the sequence which result in a silent change in the molecule so that the product retains its activity. Such amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues involved. For example, acidic amino acids (negatively charged at pH 6.0) include aspartic acid and glutamic acid; basic amino acids (positively charged at pH 6.0) include lysine and arginine; amino acids with uncharged polar head groups or nonpolar head groups having similar hydrophilic properties include the following: leucine, isoleucine, valine; glycine, alanine; asparagine, glutamine; serine, threonine; phenylalanine, tyrosine.

50 In another aspect the invention relates to processes for the production of the novel amylase of the first aspect above. According to this second aspect the amylases of the invention may be produced by the use of conventional genetic engineering techniques, such as gene splicing or by use of in vivo recombination to be described below, or by chemical synthetic techniques.

In a third aspect the invention relates to the use of the chimeric amylases in the liquefaction stage in the production of high DX syrups, especially in the jet cooking process mentioned above.

55 The chimeric alpha-amylases upon which the invention is based surprisingly demonstrate the excellent thermostability characteristics of alpha-amylase derived from B. licheniformis, but at the same time a reduced negative effect on the maximum obtainable DX without being inactivated prior to the saccharification. The invention is demonstrated herein, by way of examples, in which a segment of B. licheniformis alpha-amylase consisting of from about amino acid residue number 57 to about amino acid

residue number 87, calculated from the N-terminal end of B. licheniformis alpha-amylase or, alternatively, the whole N-terminal segment thereof, is exchanged with the corresponding segment of B. amyloliquefaciens alpha-amylase. The residual activity of the chimeric alpha-amylase has at the most a negligible negative effect on the maximum DX obtainable by saccharification with glucoamylase-PRO-MOZYME while still retaining the excellent thermostability characteristic of B. licheniformis alpha-amylase.

### 3.1 DEFINITIONS

As used herein, the following terms shall have the meanings indicated:

DS = dry substance  
DX = percentage by weight of dextrose (D-glucose).

### 4. BRIEF DESCRIPTION OF THE DRAWINGS

The invention will be described in further detail in the following specification and examples with reference to the appended drawing in which:

FIG. 1 shows gel-permeation chromatograms of alpha-amylases from B. licheniformis, B. amyloliquefaciens and an alpha-amylase according to the invention.

FIG. 2 shows the restriction map of plasmid pDN1822.

FIG. 3 shows the restriction map of plasmid pDN1850.

FIG. 4 shows the restriction map of plasmid pDN1864.

### 5. DETAILED DESCRIPTION OF THE INVENTION

As indicated above the invention relates to chimeric alpha-amylases of the general formula I

(I).

**Q-R-L**

in which Q, R, and L are defined as described in Section 3 supra. Preferred formulas are described in the subsections below.

#### 5.1 AMINO ACID SEQUENCES OF PREFERRED EMBODIMENTS

A preferred alpha-amylase of the general formula I is one in which Q is an N-terminal part of the general formula Ib:

(Ib)

**X<sub>1</sub>-Asn-Gly-Thr-Leu-Met-Gln-Tyr-Phe-Glu-Trp-Tyr-X<sub>2</sub>-Pro-Asn-Asp-**

**Gly-Gln-His-Trp-Lys-Arg-Leu-Gln-Asn-Asp-X<sub>3</sub>-Leu-X<sub>4</sub>-Gly-Ile-Thr-**

**Ala-Val-Trp-Ile-Pro-Pro-Ala-Tyr-Lys-Gly-X<sub>5</sub>-Ser-Gln-X<sub>6</sub>-Asp-X<sub>7</sub>-**

**55**

**Gly-Tyr-Gly**

in which

X<sub>1</sub> is Ala-Asn-Leu or Val,

X<sub>2</sub> is Met or Thr,

X<sub>3</sub> is Ser-Ala-Tyr or Ala-Glu-His,  
 X<sub>4</sub> is Ala-Glu-His or Ser-Asp-Ile,  
 X<sub>5</sub> is Thr or Leu,  
 X<sub>6</sub> is Ala or Ser,  
 5 X<sub>7</sub> is Val or Asn; and

R and L are defined as previously in Section 3 supra.

In another preferred alpha-amylase of the general formula I, Q and R are defined as previously described, and L is a C-terminal part of the general formula Ic

10

(Ic)

	90	95	100
15	Ser-Leu-His-Ser-Arg-Asp-Ile-Asn-Val-Tyr-Gly-Asp-Val-		
	105	110	115
	Val-Ile-Asn-His-Lys-Gly-Gly-Ala-Asp-Ala-Thr-Glu-Asp-Val-Thr-		
	120	125	130
20	Ala-Val-Glu-Val-Asp-Pro-Ala-Asp-Arg-Asn-Arg-Val-Ile-Ser-Gly-		
	135	140	145
	Glu-His-Arg-Ile-Lys-Ala-Trp-Thr-His-Phe-His-Phe-Pro-Gly-Arg-		
25	150	155	160
	Gly-Ser-Thr-Tyr-Ser-Asp-Phe-Lys-Trp-His-Trp-Tyr-His-Phe-Asp-		
	165	170	175
	Gly-Thr-Asp-Trp-Asp-Glu-Ser-Arg-Lys-Leu-Asn-Arg-Ile-Tyr-Lys-		
30	180	185	190
	Phe-Gln-Gly-Lys-Ala-Trp-Asp-Trp-Glu-Val-Ser-Asn-Glu-Asn-Gly-		

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	195	200	205
	Asn-Tyr-Asp-Tyr-Leu-Met-Tyr-Ala-Asp-Ile-Asp-Tyr-Asp-His-Pro-		
	210	215	220
5	Asp-Val-Ala-Ala-Glu-Ile-Lys-Arg-Trp-Gly-Thr-Trp-Tyr-Ala-Asn-		
	225	230	235
	Glu-Leu-Gln-Leu-Asp-Gly-Phe-Arg-Leu-Asp-Ala-Val-Lys-His-Ile-		
10	240	245	250
	Lys-Phe-Ser-Phe-Leu-Arg-Asp-Trp-Val-Asn-His-Val-Arg-Glu-Lys-		
	255	260	265
15	Thr-Gly-Lys-Glu-Met-Phe-Thr-Val-Ala-Glu-Tyr-Trp-Gln-Asn-Asp-		
	270	275	280
	Leu-Gly-Ala-Leu-Glu-Asn-Tyr-Leu-Asn-Lys-Thr-Asn-Phe-Asn-His-		
	285	290	295
20	Ser-Val-Phe-Asp-Val-Pro-Leu-His-Tyr-Gln-Phe-His-Ala-Ala-Ser-		
	300	305	319
	Thr-Gln-Gly-Gly-Gly-Tyr-Asp-Met-Arg-Lys-Leu-Leu-Asn-Ser-Thr-		
	315	320	325
25	Val-Val-Ser-Lys-His-Pro-Leu-Lys-Ala-Val-Thr-Phe-Val-Asp-Asn-		
	330	335	340
	His-Asp-Thr-Gln-Pro-Gly-Gln-Ser-Leu-Glu-Ser-Thr-Val-Gln-Thr-		
30	345	350	355
	Trp-Phe-Lys-Pro-Leu-Ala-Tyr-Ala-Phe-Ile-Leu-Thr-Arg-Glu-Ser-		
	360	365	370
	Gly-Tyr-Pro-Gln-Val-Phe-Tyr-Gly-Asp-Met-Tyr-Gly-Thr-Lys-Gly-		
35	375	380	385
	Asp-Ser-Gln-Arg-Glu-Ile-Pro-Ala-Leu-Lys-His-Lys-Ile-Glu-Pro-		
	390	395	400
40	Ile-Leu-Lys-Ala-Arg-Lys-Gln-Tyr-Ala-Tyr-Gly-Ala-Gln-His-Asp-		
	405	410	415
	Tyr-Phe-Asp-His-His-Asp-Ile-Val-Gly-Trp-Thr-Arg-Glu-Gly-Asp-		
	420	425	430
45	Ser-Ser-Val-Ala-Asn-Ser-Gly-Leu-Ala-Ala-Leu-Ile-Thr-Asp-Gly-		
	435	440	445
	Pro-Gly-Gly-Ala-Lys-Arg-Met-Tyr-Val-Gly-Arg-Gln-Asn-Ala-Gly-		

50

55

450                      455                      460  
**Glu-Thr-Trp-His-Asp-Ile -Thr-Gly-Asn-Arg-Ser-Glu-Pro -Val-Val-**  
 465                      470                      475  
 5    **Ile-Asn-Ser-Glu-Gly-Trp-Gly-Glu-Phe-His-Val-Asn-Gly-Gly-Ser-**  
          480                      483  
**Val-Ser-Ile-Tyr-Val-Gln-Arg**

10    In still another preferred alpha-amylase of the general formula I, Q has the general formula Ib and L is a C-terminal part of the general formula Ic, in which X<sub>1</sub> is Val, X<sub>2</sub> is Thr, X<sub>3</sub> is Ala-Glu-His, X<sub>4</sub> is Ser-Asp-Ile, X<sub>5</sub> is Leu, X<sub>6</sub> is Ser, and X<sub>7</sub> is Asn.

15    In yet another preferred alpha-amylase of the general formula I, Q has the general formula Ib in which X<sub>1</sub> is Val, X<sub>2</sub> is Thr, X<sub>3</sub> is Ala-Glu-His, X<sub>4</sub> is Ser-Asp-Ile, X<sub>5</sub> is Leu, X<sub>6</sub> is Ser, and X<sub>7</sub> is Asn; L is a C-terminal peptide residue of the general formula Ic; and amino acid residues X<sub>8</sub>, X<sub>9</sub>, and X<sub>10</sub> of R are Gln, Ser and Asp, respectively.

20    In yet another preferred alpha-amylase of the general formula I, Q has the general formula Ib in which Q, X<sub>1</sub> is Val, X<sub>2</sub> is Thr, X<sub>3</sub> is Ala-Glu-His, X<sub>4</sub> is Ser-Asp-Ile, X<sub>5</sub> is Leu, X<sub>6</sub> is Ser, and X<sub>7</sub> is Asn; L is a C-terminal part of the general formula Ic; and amino acid residues X<sub>8</sub>, X<sub>9</sub> and X<sub>10</sub> of R are His, Gly and Ser, respectively.

## 5.2 METHODS FOR PRODUCING THE CHIMERIC AMYLASES

25    The amylases of the invention are chimeric enzymes and may in accordance with the second aspect of the invention be produced in a number of ways as described below.

Naturally occurring enzymes may be genetically modified by random or site directed mutagenesis. Alternatively, part of one enzyme may be replaced by a part of another to obtain a chimeric enzyme. This replacement can be achieved either by conventional in vitro gene splicing techniques or by in vivo recombination or by combinations of both techniques. When using conventional in vitro gene splicing techniques, a desired portion of the alpha-amylase gene coding sequence may be deleted using appropriate site-specific restriction enzymes; the deleted portion of the coding sequence may then be replaced by the insertion of a desired portion of a different alpha-amylase coding sequence so that a chimeric nucleotide sequence encoding a new alpha-amylase is produced.

35    The in vivo recombination techniques depend on the fact that different DNA segments with highly homologous regions (identity of DNA sequence) may recombine, i.e. break and exchange DNA, and establish new bonds in the homologous regions. Accordingly, when the coding sequences for two different but homologous amylase enzymes are used to transform a host cell, recombination of homologous sequences in vivo will result in the production of chimeric gene sequences. Translation of these coding sequences by the host cell will result in production of a chimeric amylase gene product.

40    The alpha-amylase genes from Bacillus licheniformis (herein designated amyL) and from Bacillus amyloliquefaciens (herein designated amyQ) are approximately 70 percent homologous at the DNA level and suitable for hybrid formation by in vivo gene splicing.

45    In an alternate embodiment, the chimeric enzyme may be synthesized by standard chemical methods known in the art. For example, see Hunkapiller et al., 1984, Nature 310:105-111. Accordingly, peptides having the amino acid sequences described supra may be synthesized in whole or in part and joined to form the chimeric enzymes of the invention.

## 5.3 USES OF THE CHIMERIC AMYLASES

50    According to its third aspect the invention relates to the use of the novel alpha-amylases in the liquefaction stage in the overall enzymatic conversion of starch into high DX syrups.

As indicated previously residual activity from the use of the thermostable alpha-amylase from B. licheniformis in the liquefaction stage entails a negative effect on maximum obtainable D-glucose yield in the saccharification stage when using A. niger glucoamylase and B. acidopullulyticus pullulanase.

55    The reason for this negative effect is not fully understood, but it is assumed that B. licheniformis alpha-amylase generates "limit dextrins" which are poor substrates for B. acidopullulyticus pullulanase, by hydrolyzing 1, 4-alpha-glucosidic linkages close to the branch-points in amylopectin. These limit dextrins

which contain too few glucose units in one or more of the side chains will be less susceptible to B. acidopullulyticus pullulanase attack.

In FIG. 1 the action patterns for B. licheniformis alpha-amylase, B. amyloliquefaciens alpha-amylase, and the hybrid QL1864 alpha-amylase on amylopectin are indicated by the gel-permeation chromatograms taken from amylopectin digests after 48 hours.

From the figure it is seen that the action pattern of B. licheniformis alpha-amylase on amylopectin is different from that of B. amyloliquefaciens alpha-amylase. The B. licheniformis enzyme produces mainly DP<sub>6</sub>, DP<sub>5</sub> and DP<sub>3</sub> initially. On prolonged hydrolysis the DP<sub>6</sub> fraction is hydrolyzed further, and the major components are DP<sub>5</sub>, DP<sub>3</sub>, and DP<sub>2</sub>. When B. amyloliquefaciens alpha-amylase is used the major components are DP<sub>6</sub>.

The action pattern of the alpha-amylases of the invention as exemplified by the QL1864 alpha-amylase on amylopectin is distinctly different from both naturally occurring alpha-amylases, and as shown below, this changed action pattern surprisingly has resulted in the removal of the negative effect from B. licheniformis alpha-amylase on the D-glucose yield, while retaining the thermostability.

Accordingly it has been found that the alpha-amylases of the invention are very efficiently used for the liquefaction of starch.

## 6. EXAMPLE: CHIMERIC AMYLASE QL1864

The subsections below describe the production and characterization of the chimeric alpha-amylase QL1864.

### 6.1. CONSTRUCTION OF HYBRID QL1864

By conventional techniques, amyL and amyQ were cloned in B. subtilis. The restriction enzyme map of the two genes were in agreement with published DNA sequences for the genes for B. licheniformis amylase (amyL) (Stephens et al. 1986, J. Bacteriol. 158: 369 (1984)) and B. amyloliquefaciens amylase (amyQ) (Takkinen et al., 1983, J. Biol. Chem 258: 1007) 1983), respectively.

amyQ (amyQ+) and a C-terminal part of amyL (amyL-) were placed in parallel on plasmid pDN1822. This is a B. subtilis plasmid derived from cloning vector pUB110 and harbouring the chloramphenicol resistance (Cam<sup>R</sup>) gene (cat gene) of cloning vector pC194. The restriction map of pDN1822 is shown in FIG. 2, where the genes are indicated by arrows. The C-terminal part of amyQ on pDN1822 was then deleted by excision of a PvuI-PvuI fragment, which is shown hatched in FIG. 2, to obtain plasmid pDN1850 (FIG. 3). pDN1850 is amylase negative (Amy-) but harbors a N-terminal-part of amyQ and a C-terminal part of amyL. However, with a frequency of about 10<sup>-4</sup>, recombination between amyQ and amyL occurs resulting in the plasmids harbouring a hybrid QL amylase gene (amyQL+) and of an amylase positive phenotype (Amy+).

Transformation with a plasmid preparation of pDN1850 into a plasmid free B. subtilis recipient selecting for Cam<sup>R</sup> on starch containing agar plates resulted in about 1:10<sup>4</sup> transformants producing an active amylase. These transformants were surrounded by a halo of degraded starch which could be identified by iodine vapour. These Amy<sup>+</sup> transformants harboured a QL hybrid amylase gene on the plasmid. From these transformants the plasmids pDN1851 to pDN1865 were isolated, and it was found that transformants containing plasmids pDN1851, pDN1858 to pDN1862 and pDN1864 produced alpha-amylases that fulfill the objects of the invention. By restriction enzyme mapping of plasmid pDN1864, the amyQ/L1864 gene was characterized (FIG. 4) and shown to harbor an Avall site from amyQ, but not the nearby EcoRI site from amyQ. Hence, recombination between amyQ and amyL as indicated by the cross-hatched area in FIG. 3 took place between the codons coding for amino acid No. 58 and No. 67 in the B. licheniformis alpha-amylase. B. subtilis QL1864 is therefore producing a chimeric amylase composed of about 1/6 amyQ amylase at the N-terminal end and about 5/6 amyL amylase at the C-terminal end.

### 6.2 ANALYSIS OF CHIMERIC AMYLASE PRODUCED BY QL1864

In the following tests the enzyme units used are defined as indicated below:

One NU (NOVO Unit) of alpha-amylase activity is the amount of enzyme which breaks down 5.26 mg of dissolved starch per hour at 37 °C, pH 5.6 and 0.0043 M of Ca<sup>++</sup> over a 7-20 minute reaction time.

One AG unit of glucoamylase activity is the amount of enzyme which hydrolyzes one micromole of maltose per minute at 25 °C and pH 4.3.



One pullulanase unit (PUN) is defined as the amount of enzyme which under standard conditions (temperature 40 °C and pH 5.0) hydrolyzes pullulan at a rate corresponding to the formation of reducing groups equivalent to 1  $\mu$ mole of glucose per minute.

#### 5 6.2.1. SACCHARIFICATION TEST OF CHIMERIC AMYLASE

As explained above it has been found that the presence of a residual *B. licheniformis* alpha-amylase activity originating from the liquefaction stage has a negative effect on maximum D-glucose yield in the saccharification stage when *B. acidopullulyticus* pullulanase and *A. niger* glucoamylase are used in combination.

In order to evaluate the influence of a residual activity from the chimeric alpha-amylases of the invention on the saccharification stage they were compared to the *B. licheniformis* alpha-amylase in the following way:

Substrates for saccharification were prepared by redissolving a DE 8 spray-dried maltodextrin (APS 840964A) in deionized water the making up to approximately 30% DS (dry substance). Saccharification experiments were carried out in standard 500 ml laboratory batch reactions.

pH's were measured at saccharification temperature with the pH electrode and pH meter calibrated and adjusted in buffer at 60 °C.

The following standard conditions were used:

Substrate concentration	28.2% (initial)	30.8% (final)
Temperature	60 °C	
pH (initial, at 60 °C)	4.6	
Enzyme dosage:		
glucoamylase	0.15 AG/g DS	
pullulanase	0.33 PUN/g DS	
alpha-amylase	60 NU/g DS	

The results of the tests are presented in Table I

**TABLE I**  
**SACCHARIFICATION TEST OF CHIMERIC AMYLASE**

		Reaction Conditions				
		time				
		(h)	pH	%DP <sub>1</sub>	%DP <sub>2</sub>	%DP <sub>3</sub>
						%DP <sub>4</sub>
10	<b>Alpha-Amylase</b>					
	None	24	4.5	92.8	2.5	1.1
	(Control)	48	4.4	96.7	1.8	0.7
		72	4.4	96.8	2.0	0.6
15		96	4.4	96.8	2.2	0.5
20	<b><u>B. licheniformis</u></b>	24	4.5	92.4	2.5	2.4
		48	4.5	95.9	1.8	1.5
		72	4.4	96.2	2.0	1.1
		96	4.4	96.4	2.1	0.9
25	<b>QL 1864</b>	24	4.6	92.1	2.8	1.9
		48	4.5	96.3	1.7	1.2
		72	4.5	96.5	2.0	0.9
		96	4.5	96.6	2.1	0.8
30						

From the results shown in Table I it is seen that although the presence of QL 1864 alpha-amylase slightly reduced the maximum obtainable DX (in comparison to the control), it represents a significant improvement over the B. licheniformis alpha-amylase.

#### 6.2.2 THERMOACTIVATION OF CHIMERIC AMYLASE

In order to evaluate the thermoactivation of the chimeric alpha-amylases produced by the transformed strains the chimeric alpha-amylases were submitted to the following test:

Substrate: Phadebas tables (Phadebas® amylase test, Pharmacia Diagnostics, Sweden) a cross-linked blue coloured starch polymer insoluble in water.  
 Buffer: 0.1 M phosphate, pH 6.1, and TRIS buffer pH 9.5.  
 Enzyme: alpha-Amylase diluted to 1-2 NU/ml in 0.09 M CaCl<sub>2</sub>, pH 6.1.  
 Temperatures: 37°C and 85°C

1 ml alpha-amylase dilution was thoroughly mixed with 5 ml buffer and incubated in a water bath at the desired temperature prior to the addition of one Phadebas table.

The test tube was shaken for 15 seconds on a whirl mixer before it is placed in the water bath again.

After exactly 15 minutes the reaction was stopped by the addition of 1 ml 1 M NaOH. After mixing the mixture was filtered through a 9 cm Whatman® GF/A or FG/C filter.

The optical density of the filtrate was measured at a wavelength of 620 nm, and was found to be linearly related to the activity of alpha-amylase added.

The results are presented in Table II below together with values from tests with pure B. licheniformis and B. amyloliquefaciens alpha-amylases.

**TABLE II**  
**THERMOACTIVATION OF CHIMERIC AMYLASE**

	Phadebas 37°C	
	pH 6.1:pH 9.5	Phadebas pH 6.1 75°C:37°C
<b>Alpha-Amylase</b>		
<b><u>B. licheniformis</u></b>	<b>0.4</b>	<b>3.7</b>
<b>(control)</b>		
<b>QL1864</b>	<b>2.5</b>	<b>2.5</b>
<b>QL1861</b>	<b>2.2</b>	<b>2.2</b>
<b>QL1851</b>	<b>2.1</b>	<b>2.1</b>
<b>QL1862</b>	<b>2.0</b>	<b>2.0</b>
<b>QL1858</b>	<b>2.0</b>	<b>2.0</b>
<b><u>B. amyloliquefaciens</u></b>	<b>8.7</b>	<b>0.01</b>
<b>(control)</b>		

The data presented in Table II demonstrate that the chimeric alpha-amylases of the invention are as thermoactivated as the B. licheniformis alpha-amylase, and less sensitive to alkaline pH than the B. amyloliquefaciens alpha-amylase.

#### 6.2.3. THERMOSTABILITY OF CHIMERIC AMYLASE

In order to evaluate the stability of the alpha-amylases of the invention the following steel tube tests were performed:

A DE 7 maltodextrin redissolved in deionized water was used as substrate under the following conditions:

Substrate: 32 - 33 percent  
alpha-amylase dosage: 120 NU/g maltodextrin  
Temperature: 105°C  
pH: 5.5  
Calcium content: 60 ppm

In each test 5 steel tubes containing the above reaction mixture were placed in an oil bath at 105°C and taken out after 10, 20, 30, 40, and 60 minutes, respectively, and the residual alpha-amylase activity measured by the Phadebas method described above. The half life,  $T_{1/2}$ , is calculated by linear regression of log (residual activity) versus time. The results are shown in Table III below.

**TABLE III**

THERMOSTABILITY OF CHIMERIC AMYLASES	
Alpha-Amylase	$T_{1/2}$ minutes
<b><u>B. amyloliquefaciens</u> (control)</b>	<b>5</b>
<b>QL 1851</b>	<b>22</b>
<b>QL 1858</b>	<b>25</b>
<b>QL 1861</b>	<b>18</b>
<b>QL 1862</b>	<b>22</b>
<b>QL 1864</b>	<b>24</b>
<b><u>B. licheniformis</u> (control)</b>	<b>23</b>

From the results shown in Table III it is clearly seen that the hybrid alpha-amylases of the invention have retained the stability of the B. licheniformis alpha-amylase.

#### Claims

5 **Claims for the following Contracting States : BE, CH, DE, FR, GB, IT, LI, LU, NL, SE**

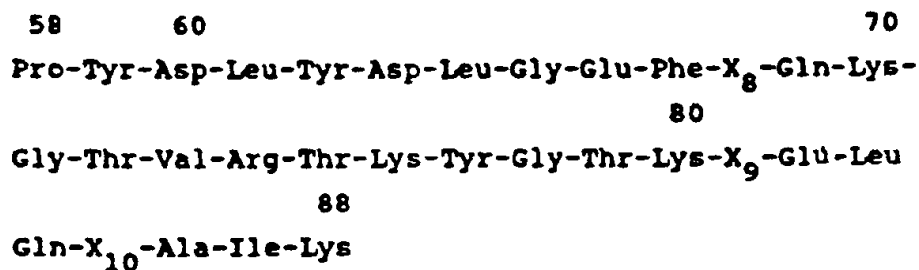
1. A chimeric alpha-amylase, which is thermostable and exhibits a reduced negative effect on the use of A. niger glucoamylase and B. acidopullulyticus pullulanase for the saccharification of starch, having the general formula I

#### (I) Q-R-L

in which Q comprises a N-terminal part of from 55 to 60 amino acid residues which is at least 75% homologous to the 55 N-terminal amino acid residues in the Bacillus amyloliquefaciens alpha-amylase as described in Takkinen et al., J. Biol. Chem. 258 (1983) 1007-1013;

R comprises a part of the general formula Ia

#### (Ia)



in which

- X<sub>8</sub> comprises His or Gln,  
 X<sub>9</sub> comprises Gly or Ser,  
 X<sub>10</sub> comprises Ser or Asp; and

L comprises a C-terminal part of from 390 to 400 amino acid residues which is at least 75% homologous to the 395 C-terminal amino acid residues in the Bacillus licheniformis 584 (ATCC 27811) alpha-amylase.

2. The chimeric alpha-amylase according to claim 1, in which

- X<sub>8</sub> comprises His,  
 X<sub>9</sub> comprises Gly, and  
 X<sub>10</sub> comprises Ser.

3. The chimeric alpha-amylase according to claim 1, in which

- X<sub>8</sub> comprises Gln,  
 X<sub>9</sub> comprises Ser, and  
 X<sub>10</sub> comprises Asp.

4. The chimeric alpha-amylase according to claim 1, in which the homologies are at least 80 percent.

5. The chimeric alpha-amylase according to claim 1, in which the homologies are at least 90 percent.

6. The chimeric alpha-amylase according to claim 1, in which Q comprises an N-terminal part of the general formula Ib

(Ib)

5                    5                    10                    15  
 5 **X<sub>1</sub>-Asn-Gly-Thr-Leu-Met-Gln-Tyr-Phe-Glu-Trp-Tyr-X<sub>2</sub>-Pro-Asn-**  
                   20                    25                    30                    35  
**Asp-Gly-Gln-His-Trp-Lys-Arg-Leu-Gln-Asn-Asp-X<sub>3</sub>-Leu-X<sub>4</sub>-Gly-**  
 10                    40                    45                    50  
**Ile-Thr-Ala-Val-Trp-Ile-Pro-Pro-Ala-Tyr-Lys-Gly-X<sub>5</sub>-Ser-Gln-**  
                   55  
 15 **X<sub>6</sub>-Asp-X<sub>7</sub>-Gly-Tyr-Gly;**

in which

- X<sub>1</sub> comprises Ala-Asn-Leu or Val,  
 X<sub>2</sub> comprises Met or Thr,  
 20 X<sub>3</sub> comprises Ser-Ala-Tyr or Ala-Glu-His,  
 X<sub>4</sub> comprises Ala-Gly-His or Ser-Asp-Ile,  
 X<sub>5</sub> comprises Thr or Leu,  
 X<sub>6</sub> comprises Ala or Ser, and  
 X<sub>7</sub> comprises Val or Asn.

25

7. The chimeric alpha-amylase according to claim 6, in which

- X<sub>1</sub> comprises Val,  
 X<sub>2</sub> comprises Thr,  
 X<sub>3</sub> comprises Ala-Glu-His,  
 30 X<sub>4</sub> comprises Ser-Asp-Ile,  
 X<sub>5</sub> comprises Leu,  
 X<sub>6</sub> comprises Ser, and  
 X<sub>7</sub> comprises Asn.

- 35 8. The alpha-amylase according to claim 4, 5, 6, or 7, in which L comprises a C-terminal part of the general formula Ic

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(Ic)

5                   90                               95                               100  
           Ser-Leu-His-Ser-Arg-Asp-Ile-Asn-Val-Tyr-Gly-Asp-Val  
 10                   105                               110                               115  
           Val-Ile-Asn-His-Lys-Gly-Gly-Ala-Asp-Ala-Thr-Glu-Asp-Val-Thr-  
 15                   120                               125                               130  
           Ala-Val-Glu-Val-Asp-Pro-Ala-Asp-Arg-Asn-Arg-Val-Ile-Ser-Gly-  
           135                               140                               145  
           Glu-His-Arg-Ile-Lys-Ala-Trp-Thr-His-Phe-His-Phe-Pro-Gly-Arg-  
 20                   150                               155                               160  
           Gly-Ser-Thr-Tyr-Ser-Asp-Phe-Lys-Trp-His-Trp-Tyr-His-Phe-Asp-  
           165                               170                               175  
           Gly-Thr-Asp-Trp-Asp-Glu-Ser-Arg-Lys-Leu-Asn-Arg-Ile-Tyr-Lys-  
 25                   180                               185                               190  
           Phe-Gln-Gly-Lys-Ala-Trp-Asp-Trp-Glu-Val-Ser-Asn-Glu-Asn-Gly-  
           195                               200                               205  
           Asn-Tyr-Asp-Tyr-Leu-Met-Tyr-Ala-Asp-Ile-Asp-Tyr-Asp-His-Pro-  
           210                               215                               220  
           Asp-Val-Ala-Ala-Glu-Ile-Lys-Arg-Trp-Gly-Thr-Trp-Tyr-Ala-Asn-  
 30                   225                               230                               235  
           Glu-Leu-Gln-Leu-Asp-Gly-Phe-Arg-Leu-Asp-Ala-Val-Lys-His-Ile-  
           240                               245                               250  
           Lys-Phe-Ser-Phe-Leu-Arg-Asp-Trp-Val-Asn-His-Val-Arg-Glu-Lys-  
 35                   255                               260                               265  
           Thr-Gly-Lys-Glu-Met-Phe-Thr-Val-Ala-Glu-Tyr-Trp-Gln-Asn-Asp-  
           270                               275                               280  
           Leu-Gly-Ala-Leu-Glu-Asn-Tyr-Leu-Asn-Lys-Thr-Asn-Phe-Asn-His-  
           285                               290                               285  
           Ser-Val-Phe-Asp-Val-Pro-Leu-His-Tyr-Gln-Phe-His-Ala-Ala-Ser-  
 45                   300                               305                               310  
           Thr-Gln-Gly-Gly-Gly-Tyr-Asp-Met-Arg-Lys-Leu-Leu-Asn-Ser-Thr-  
 50  
 55

- 15

- (I)

in which Q comprises a N-terminal part of from 55 to 60 amino acid residues which is at least 75% homologous to the 55 N-terminal amino acid residues in the *Bacillus amyloliquefaciens* alpha-amylase as described in Takkinen et al., J. Biol. Chem. 258 (1983) 1007-1013;

(Id)

in which

**X<sub>8</sub>** comprises His or Gln,

X<sub>9</sub> comprises Gly or Ser,

**X<sub>10</sub>** comprises Ser or Asp; and

L comprises a C-terminal part of from 390 to 400 amino acid residues which is at least 75% homologous to the 395 C-terminal amino acid residues in the Bacillus licheniformis 584 (ATCC 27811) alpha-amylase, to form oligosaccharides; and

(b) reacting the oligosaccharides formed in step (a) with a glucoamylase to form dextrose.

- 3. A process according to Claim 2, wherein, in the chimeric alpha-amylase,**

**X<sub>8</sub>** comprises His,

X<sub>9</sub> comprises Gly, and

$X_{10}$  comprises Ser.

4. A process according to Claim 2, wherein, in the chimeric alpha-amylase

$X_8$  comprises Gln.

X<sub>9</sub> comprises Ser<sub>1</sub> and

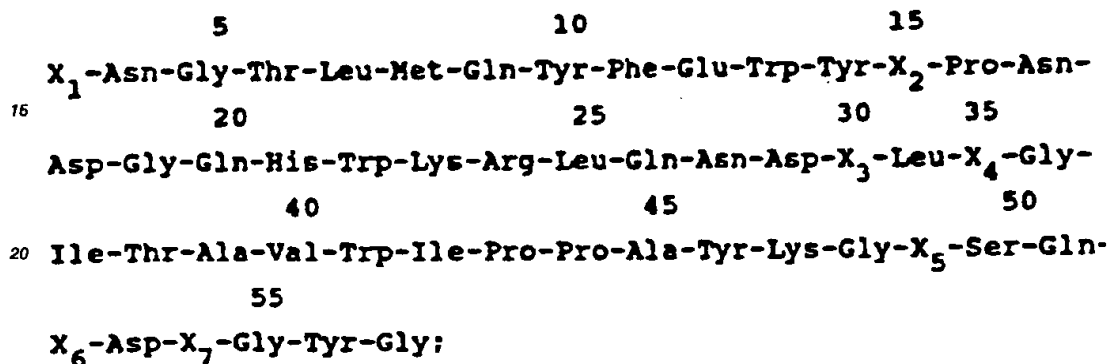
X<sub>10</sub> comprises Asp.



5. A process according to Claim 2, wherein, in the chimeric alpha-amylase, the homologies are at least 80 percent.
6. A process according to Claim 2, wherein, in the chimeric alpha-amylase the homologies are at least 90 percent.
7. A process according to Claim 2, wherein, in the chimeric alpha-amylase, Q comprises an N-terminal part of the general formula Ib

10

(Ib)



25

in which:

- X<sub>1</sub> comprises Ala-Asn-Leu or Val,
- X<sub>2</sub> comprises Met or Thr,
- X<sub>3</sub> comprises Ser-Ala-Tyr or Ala-Glu-His,
- 30 X<sub>4</sub> comprises Ala-Gly-His or Ser-Asp-Ile,
- X<sub>5</sub> comprises Thr or Leu,
- X<sub>6</sub> comprises Ala or Ser, and
- X<sub>7</sub> comprises Val or Asn.

35

8. A process according to Claim 7, wherein, in the chimeric alpha-amylase,

- X<sub>1</sub> comprises Val,
- X<sub>2</sub> comprises Thr,
- X<sub>3</sub> comprises Ala-Glu-His,
- X<sub>4</sub> comprises Ser-Asp-Ile,
- 40 X<sub>5</sub> comprises Leu,
- X<sub>6</sub> comprises Ser, and
- X<sub>7</sub> comprises Asn.

45

9. A process for converting starch into high dextrose syrup, comprising:

- (a) reacting the starch with a chimeric alpha-amylase to form oligosaccharides; and
- (b) reacting the oligosaccharides formed in step (a) with a glucoamylase to form dextrose, the chimeric alpha-amylase being as defined in Claim 5, 6, 7 or 8 and in which L comprises a C-terminal part of the general formula Ic

50

55

(Ic)

5                   90                                   95                                   100  
           Ser-Leu-His-Ser-Arg-Asp-Ile-Asn-Val-Tyr-Gly-Asp-Val  
  
                   105                                   110                                   115  
 Val-Ile-Asn-His-Lys-Gly-Gly-Ala-Asp-Ala-Thr-Glu-Asp-Val-Thr-  
  
 10                   120                                   125                                   130  
 Ala-Val-Glu-Val-Asp-Pro-Ala-Asp-Arg-Asn-Arg-Val-Ile-Ser-Gly-  
  
                   135                                   140                                   145  
 Glu-His-Arg-Ile-Lys-Ala-Trp-Thr-His-Phe-His-Phe-Pro-Gly-Arg-  
  
 15                   150                                   155                                   160  
 Gly-Ser-Thr-Tyr-Ser-Asp-Phe-Lys-Trp-His-Trp-Tyr-His-Phe-Asp-  
  
                   165                                   170                                   175  
 20 Gly-Thr-Asp-Trp-Asp-Glu-Ser-Arg-Lys-Leu-Asn-Arg-Ile-Tyr-Lys-  
  
                   180                                   185                                   190  
 Phe-Gln-Gly-Lys-Ala-Trp-Asp-Trp-Glu-Val-Ser-Asn-Glu-Asn-Gly-  
  
 25                   195                                   200                                   205  
 Asn-Tyr-Asp-Tyr-Leu-Met-Tyr-Ala-Asp-Ile-Asp-Tyr-Asp-His-Pro-  
  
                   210                                   215                                   220  
 Asp-Val-Ala-Ala-Glu-Ile-Lys-Arg-Trp-Gly-Thr-Trp-Tyr-Ala-Asn-  
  
 30                   225                                   230                                   235  
 Glu-Leu-Gln-Leu-Asp-Gly-Phe-Arg-Leu-Asp-Ala-Val-Lys-His-Ile-  
  
                   240                                   245                                   250  
 35 Lys-Phe-Ser-Phe-Leu-Arg-Asp-Trp-Val-Asn-His-Val-Arg-Glu-Lys-  
  
                   255                                   260                                   265  
 Thr-Gly-Lys-Glu-Met-Phe-Thr-Val-Ala-Glu-Tyr-Trp-Gln-Asn-Asp-  
  
 40                   270                                   275                                   280  
 Leu-Gly-Ala-Leu-Glu-Asn-Tyr-Leu-Asn-Lys-Thr-Asn-Phe-Asn-His-  
  
                   285                                   290                                   285  
 Ser-Val-Phe-Asp-Val-Pro-Leu-His-Tyr-Gln-Phe-His-Ala-Ala-Ser-  
  
 45                   300                                   305                                   310  
 Thr-Gln-Gly-Gly-Gly-Tyr-Asp-Met-Arg-Lys-Leu-Leu-Asn-Ser-Thr-  
  
 50  
  
 55

315 320 325  
 Val-Val-Ser-Lys-His-Pro-Leu-Lys-Ala-Val-Thr-Phe-Val-Asp-Asn-  
 5 His-Asp-Thr-Gln-Pro-Gly-Gln-Ser-Leu-Glu-Ser-Thr-Val-Gln-Thr-  
 330 335 340  
 Trp-Phe-Lys-Pro-Leu-Ala-Tyr-Ala-Phe-Ile-Leu-Thr-Arg-Glu-Ser-  
 10 345 350 355  
 Gly-Tyr-Pro-Gln-Val-Phe-Tyr-Gly-Asp-Met-Tyr-Gly-Thr-Lys-Gly-  
 360 365 370  
 Asp-Ser-Gln-Arg-Glu-Ile-Pro-Ala-Leu-Lys-His-Lys-Ile-Glu-Pro-  
 15 375 380 385  
 Ile-Leu-Lys-Ala-Arg-Lys-Gln-Tyr-Ala-Tyr-Gly-Ala-Gln-His-Asp-  
 390 395 400  
 Tyr-Phe-Asp-His-His-Asp-Ile-Val-Gly-Trp-Thr-Arg-Glu-Gly-Asp-  
 20 405 410 415  
 Ser-Ser-Val-Ala-Asn-Ser-Gly-Leu-Ala-Ala-Leu-Ile-Thr-Asp-Gly-  
 420 425 430  
 25 435 440 445  
 Pro-Gly-Gly-Ala-Lys-Arg-Met-Tyr-Val-Gly-Arg-Gln-Asn-Ala-Gly-  
 450 455 460  
 Glu-Thr-Trp-His-Asp-Ile-Thr-Gly-Asn-Arg-Ser-Glu-Pro-Val-Val-  
 30 465 470 475  
 Ile-Asn-Ser-Glu-Gly-Trp-Gly-Glu-Phe-His-Val-Asn-Gly-Gly-Ser-  
 480 483  
 35 Val-Ser-Ile-Tyr-Val-Gln-Arg.

#### Patentansprüche

40 Patentansprüche für folgende Vertragsstaaten : BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Chimäre Alpha-Amylase, die hitzestabil ist und eine verringerte negative Wirkung auf die Verwendung von Glucoamylase aus A. niger und Pullulanase aus B. acidopullulyticus zur Verzuckerung von Stärke zeigt, mit der allgemeinen Formel I

45

(I)

Q-R-L,

50

in der Q eine N-terminalen Teil mit von 55 bis 60 Aminosäureresten umfaßt, der wenigstens 75% homolog zu den 55 N-terminalen Aminosäureresten in der Alpha-Amylase aus Bacillus amyloliquefaciens ist, wie in Takkinen et al., J. Biol. Chem. 258 (1983), 1007-1013 beschrieben;

R einen Teil der allgemeinen Formel Ia umfaßt

55

15 in der  
X<sub>8</sub> His oder Gln umfaßt,  
X<sub>9</sub> Gly oder Ser umfaßt,  
X<sub>10</sub> Ser oder Asp umfaßt; und  
20 L einen C-terminalen Teil mit von 390 bis 400 Aminosäureresten umfaßt, der wenigstens 75% homolog  
zu den 395 C-terminalen Aminosäureresten in der Alpha-Amylase aus Bacillus licheniformis 584 (ATCC  
27811) ist.

2. Chimäre Alpha-Amylase nach Anspruch 1, in der  
25 X<sub>8</sub> His umfaßt,  
X<sub>9</sub> Gly umfaßt, und  
X<sub>10</sub> Ser umfaßt.
3. Chimäre Alpha-Amylase nach Anspruch 1, in der  
30 X<sub>8</sub> Gln umfaßt,  
X<sub>9</sub> Ser umfaßt und  
X<sub>10</sub> Asp umfaßt.
4. Chimäre Alpha-Amylase nach Anspruch 1, in der die Homologien wenigstens 80 Prozent sind.
5. Chimäre Alpha-Amylase nach Anspruch 1, in der die Homologien wenigstens 90 Prozent sind.
6. Chimäre Alpha-Amylase nach Anspruch 1, in der Q einen N-terminalen Teil der allgemeinen Formel Ib  
35 umfaßt

(Ib)

in der  
X<sub>1</sub> Ala-Asn-Leu oder Val umfaßt,

- 5  
X<sub>2</sub> Met oder Thr umfaßt,  
X<sub>3</sub> Ser-Ala-Tyr oder Ala-Glu-His umfaßt,  
X<sub>4</sub> Ala-Gly-His oder Ser-Asp-Ile umfaßt,  
X<sub>5</sub> Thr oder Leu umfaßt,  
X<sub>6</sub> Ala oder Ser umfaßt und  
X<sub>7</sub> Val oder Asn umfaßt.

7. Chimäre Alpha-Amylase nach Anspruch 6, in der  
10 X<sub>1</sub> Val umfaßt,  
X<sub>2</sub> Thr umfaßt,  
X<sub>3</sub> Ala-Glu-His umfaßt,  
X<sub>4</sub> Ser-Asp-Ile umfaßt,  
X<sub>5</sub> Leu umfaßt,  
X<sub>6</sub> Ser umfaßt und  
15 X<sub>7</sub> Asn umfaßt.

8. Alpha-Amylase nach Anspruch 4, 5, 6 oder 7, in der L einen C-terminalen Teil der allgemeinen Formel  
Ic umfaßt

20

(Ic)

25                   90                                   95                                   100  
          Ser-Leu-His-Ser-Arg-Asp-Ile-Asn-Val-Tyr-Gly-Asp-Val  
  
                  105                                   110                                   115  
Val-Ile-Asn-His-Lys-Gly-Gly-Ala-Asp-Ala-Thr-Glu-Asp-Val-Thr-  
30                   120                                   125                                   130  
Ala-Val-Glu-Val-Asp-Pro-Ala-Asp-Arg-Asn-Arg-Val-Ile-Ser-Gly-  
  
                  135                                   140                                   145  
35   Glu-His-Arg-Ile-Lys-Ala-Trp-Thr-His-Phe-His-Phe-Pro-Gly-Arg-  
  
                  150                                   155                                   160  
Gly-Ser-Thr-Tyr-Ser-Asp-Phe-Lys-Trp-His-Trp-Tyr-His-Phe-Asp-  
40                   165                                   170                                   175  
Gly-Thr-Asp-Trp-Asp-Glu-Ser-Arg-Lys-Leu-Asn-Arg-Ile-Tyr-Lys-

45

50

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180 185 190  
 Phe-Gln-Gly-Lys-Ala-Trp-Asp-Trp-Glu-Val-Ser-Asn-Glu-Asn-Gly-  
 195 200 205  
 5 Asn-Tyr-Asp-Tyr-Leu-Met-Tyr-Ala-Asp-Ile-Asp-Tyr-Asp-His-Pro-  
 210 215 220  
 Asp-Val-Ala-Ala-Glu-Ile-Lys-Arg-Trp-Gly-Thr-Trp-Tyr-Ala-Asn-  
 10 225 230 235  
 Glu-Leu-Gln-Leu-Asp-Gly-Phe-Arg-Leu-Asp-Ala-Val-Lys-His-Ile-  
 240 245 250  
 Lys-Phe-Ser-Phe-Leu-Arg-Asp-Trp-Val-Asn-His-Val-Arg-Glu-Lys-  
 15 255 260 265  
 Thr-Gly-Lys-Glu-Met-Phe-Thr-Val-Ala-Glu-Tyr-Trp-Gln-Asn-Asp-  
 270 275 280  
 Leu-Gly-Ala-Leu-Glu-Asn-Tyr-Leu-Asn-Lys-Thr-Asn-Phe-Asn-His-  
 20 285 290 285  
 Ser-Val-Phe-Asp-Val-Pro-Leu-His-Tyr-Gln-Phe-His-Ala-Ala-Ser-  
 300 305 310  
 25 Thr-Gln-Gly-Gly-Gly-Tyr-Asp-Met-Arg-Lys-Leu-Leu-Asn-Ser-Thr-  
 315 320 325  
 Val-Val-Ser-Lys-His-Pro-Leu-Lys-Ala-Val-Thr-Phe-Val-Asp-Asn-  
 330 335 340  
 30 His-Asp-Thr-Gln-Pro-Gly-Gln-Ser-Leu-Glu-Ser-Thr-Val-Gln-Thr-  
 345 350 355  
 Trp-Phe-Lys-Pro-Leu-Ala-Tyr-Ala-Phe-Ile-Leu-Thr-Arg-Glu-Ser-  
 35 360 365 370  
 Gly-Tyr-Pro-Gln-Val-Phe-Tyr-Gly-Asp-Met-Tyr-Gly-Thr-Lys-Gly-  
 375 380 385  
 Asp-Ser-Gln-Arg-Glu-Ile-Pro-Ala-Leu-Lys-His-Lys-Ile-Glu-Pro-  
 40 390 395 400  
 Ile-Leu-Lys-Ala-Arg-Lys-Gln-Tyr-Ala-Tyr-Gly-Ala-Gln-His-Asp-  
 405 410 415  
 Tyr-Phe-Asp-His-His-Asp-Ile-Val-Gly-Trp-Thr-Arg-Glu-Gly-Asp-  
 45 420 425 430  
 Ser-Ser-Val-Ala-Asn-Ser-Gly-Leu-Ala-Ala-Leu-Ile-Thr-Asp-Gly-  
 435 440 445  
 50 Pro-Gly-Gly-Ala-Lys-Arg-Met-Tyr-Val-Gly-Arg-Gln-Asn-Ala-Gly-  
 55

450
455
460  
 Glu-Thr-Trp-His-Asp-Ile-Thr-Gly-Asn-Arg-Ser-Glu-Pro-Val-Val-  
465
470
475  
 5 Ile-Asn-Ser-Glu-Gly-Trp-Gly-Glu-Phe-His-Val-Asn-Gly-Gly-Ser-  
480
483  
 Val-Ser-Ile-Tyr-Val-Gln-Arg.

10

9. Verfahren zur Herstellung einer chimären Alpha-Amylase, welches umfaßt:
  - (a) daß die N-terminale Kodierungsregion des Alpha-Amylase-Gens von B. amyloliquefaciens mit der C-terminalen Kodierungsregion des Alpha-Amylase-Gens von B. licheniformis in vivo rekombiniert wird, um Rekombinanten zu bilden;
  - (b) daß die Rekombinanten, die eine chimäre Alpha-Amylase produzieren, die hitzestabil ist und eine verringerte negative Wirkung auf die Verwendung von Glucoamylase aus A. niger und Pullulanase aus B. acidopullulyticus zur Verzuckerung von Stärke zeigt, selektiert werden;
  - (c) daß die selektierten Rekombinanten in einem geeigneten Wachstumsmedium kultiviert werden und
  - (d) daß die chimäre Alpha-Amylase aus der Kultur gewonnen wird.
10. Verfahren zur Umwandlung von Stärke in High-Dextrose-Sirup, welches umfaßt:
  - (a) daß die Stärke mit der chimären Alpha-Amylase von Anspruch 1, 2, 3, 4, 5, 6 oder 7 zur Reaktion gebracht wird, um Oligosaccharide zu bilden; und
  - (b) daß die in Schritt (a) gebildeten Oligosaccharide mit einer Glucoamylase zur Reaktion gebracht werden, um Dextrose zu bilden.
11. Verfahren zur Umwandlung von Stärke in High-Dextrose-Sirup, welches umfaßt:
  - (a) daß die Stärke mit der chimären Alpha-Amylase von Anspruch 8 zur Reaktion gebracht wird, um Oligosaccharide zu bilden; und
  - (b) daß die in Schritt (a) gebildeten Oligosaccharide mit einer Glucoamylase zur Reaktion gebracht werden, um Dextrose zu bilden.

35 **Patentansprüche für folgende Vertragsstaaten : AT, ES, GR**

1. Verfahren zur Herstellung einer chimären Alpha-Amylase, welches umfaßt:
  - a) daß die N-terminale Kodierungsregion des Alpha-Amylase-Gens von B. amyloliquefaciens mit der C-terminalen Kodierungsregion des Alpha-Amylase-Gens von B. licheniformis in vivo rekombiniert wird, um Rekombinanten zu bilden;
  - b) daß die Rekombinanten, die eine chimäre Alpha-Amylase produzieren, die hitzestabil ist und eine verringerte negative Wirkung auf die Verwendung von Glucoamylase aus A. niger und Pullulanase aus B. acidopullulyticus zur Verzuckerung von Stärke zeigt, selektiert werden;
  - c) daß die selektierten Rekombinanten in einem geeigneten Wachstumsmedium kultiviert werden und
  - d) daß die chimäre Alpha-Amylase aus der Kultur gewonnen wird.
2. Verfahren zur Umwandlung von Stärke in High-Dextrose-Sirup, welches umfaßt:
  - a) daß die Stärke mit einer chimären Alpha-Amylase zur Reaktion gebracht wird, die hitzestabil ist und eine verringerte negative Wirkung auf die Verwendung von Glucoamylase aus A. niger und Pullulanase aus B. acidopullulyticus zur Verzuckerung von Stärke zeigt, mit der allgemeinen Formel

55

( I )  
Q-R-L

in der Q einen N-terminalen Teil mit von 55 bis 60 Aminosäureresten umfaßt, der wenigstens 75% homolog zu den 55 N-terminalen Aminosäureresten in der Alpha-Amylase aus *Bacillus amyloliquefaciens* ist, wie in Takikine et al., J. Biol. Chem. 258 (1983), 1007-1013 beschrieben;  
R einen Teil der allgemeinen Formel Ia umfaßt

(Ia)

[illegible]

in der

X<sub>8</sub> His oder Gln umfaßt,

X<sub>9</sub> Gly oder Ser umfaßt,

X<sub>10</sub> Ser oder Asp umfaßt; und

L einen C-terminalen Teil mit von 390 bis 400 Aminosäureresten umfaßt, der wenigstens 75% homolog zu den 395 C-terminalen Aminosäureresten in der Alpha-Amylase aus Bacillus licheniformis 584 (ATCC 27811) ist, um Oligosaccharide zu bilden; und

(b) daß die in Schritt (a) gebildeten Oligosaccharide mit einer Glucoamylase zur Reaktion gebracht werden, um Dextrose zu bilden.

- 3. Verfahren nach Anspruch 2, dadurch gekennzeichnet, daß in der chimären Alpha-Amylase**

**X<sub>8</sub>**      His umfaßt,

X<sub>9</sub> Gly umfaßt und

**X<sub>10</sub>** Ser umfaßt.

4. Verfahren nach Anspruch 2, dadurch gekennzeichnet, daß in der chimären Alpha-Amylase

X<sub>8</sub> Gln umfaßt,

X<sub>9</sub> Ser umfaßt und

X<sub>10</sub> Asp umfaßt.

5. Verfahren nach Anspruch 2, dadurch gekennzeichnet, daß in der chimären Alpha-Amylase die Homologien wenigstens 80% sind.

6. Verfahren nach Anspruch 2, dadurch gekennzeichnet, daß in der chimären Alpha-Amylase die Homologien wenigstens 90% sind.

7. Verfahren nach Anspruch 2, dadurch gekennzeichnet, daß in der chimären Alpha-Amylase Q einen N-terminalen Teil der allgemeinen Formel Ib umfaßt



(Ib)

5                    5                    10                    15  
 10    **X<sub>1</sub>-Asn-Gly-Thr-Leu-Met-Gln-Tyr-Phe-Glu-Trp-Tyr-X<sub>2</sub>-Pro-Asn-**  
                   20                    25                    30                    35  
 10    **Asp-Gly-Gln-His-Trp-Lys-Arg-Leu-Gln-Asn-Asp-X<sub>3</sub>-Leu-X<sub>4</sub>-Gly-**  
                   40                    45                    50  
                   55  
 15    **X<sub>6</sub>-Asp-X<sub>7</sub>-Gly-Tyr-Gly:**

in der

20    X<sub>1</sub>    Ala-Asn-Leu oder Val umfaßt,  
       X<sub>2</sub>    Met oder Thr umfaßt,  
       X<sub>3</sub>    Ser-Ala-Tyr oder Ala-Glu-His umfaßt,  
       X<sub>4</sub>    Ala-Gly-His oder Ser-Asp-Ile umfaßt,  
       X<sub>5</sub>    Thr oder Leu umfaßt,  
       X<sub>6</sub>    Ala oder Ser umfaßt und  
 25    X<sub>7</sub>    Val oder Asn umfaßt.

8. Verfahren nach Anspruch 7, dadurch gekennzeichnet, daß in der chimären Alpha-Amylase

30    X<sub>1</sub>    Val umfaßt,  
       X<sub>2</sub>    Thr umfaßt,  
       X<sub>3</sub>    Ala-Glu-His umfaßt,  
       X<sub>4</sub>    Ser-Asp-Ile umfaßt,  
       X<sub>5</sub>    Leu umfaßt,  
       X<sub>6</sub>    Ser umfaßt und  
 35    X<sub>7</sub>    Asn umfaßt.

9. Verfahren zur Umwandlung von Stärke in High-Dextrose-Sirup, welches umfaßt:

40    (a) daß die Stärke mit einer chimären Alpha-Amylase zur Reaktion gebracht wird, um Oligosaccharide zu bilden; und  
       (b) daß die in Schritt (a) gebildeten Oligosaccharide mit einer Glucoamylase zur Reaktion gebracht werden, um Dextrose zu bilden, wobei die chimäre Alpha-Amylase so ist, wie in Anspruch 5, 6, 7 oder 8 definiert, und in der L einen C-terminalen Teil der allgemeinen Formel Ic umfaßt

46

50

55

(Ic)

5                   90                   95                   100  
           Ser-Leu-His-Ser-Arg-Asp-Ile-Asn-Val-Tyr-Gly-Asp-Val  
                   105                   110                   115  
 10 Val-Ile-Asn-His-Lys-Gly-Gly-Ala-Asp-Ala-Thr-Glu-Asp-Val-Thr-  
                   120                   125                   130  
           Ala-Val-Glu-Val-Asp-Pro-Ala-Asp-Arg-Asn-Arg-Val-Ile-Ser-Gly-  
 15                   135                   140                   145  
           Glu-His-Arg-Ile-Lys-Ala-Trp-Thr-His-Phe-His-Phe-Pro-Gly-Arg-  
                   150                   155                   160  
           Gly-Ser-Thr-Tyr-Ser-Asp-Phe-Lys-Trp-His-Trp-Tyr-His-Phe-Asp-  
 20                   165                   170                   175  
           Gly-Thr-Asp-Trp-Asp-Glu-Ser-Arg-Lys-Leu-Asn-Arg-Ile-Tyr-Lys-  
           -----  
 25                   180                   185                   190  
           Phe-Gln-Gly-Lys-Ala-Trp-Asp-Trp-Glu-Val-Ser-Asn-Glu-Asn-Gly-  
                   195                   200                   205  
 30            Asn-Tyr-Asp-Tyr-Leu-Met-Tyr-Ala-Asp-Ile-Asp-Tyr-Asp-His-Pro-  
                   210                   215                   220  
           Asp-Val-Ala-Ala-Glu-Ile-Lys-Arg-Trp-Gly-Thr-Trp-Tyr-Ala-Asn-  
                   225                   230                   235  
 35            Glu-Leu-Gln-Leu-Asp-Gly-Phe-Arg-Leu-Asp-Ala-Val-Lys-His-Ile-  
                   240                   245                   250  
           Lys-Phe-Ser-Phe-Leu-Arg-Asp-Trp-Val-Asn-His-Val-Arg-Glu-Lys-  
 40                   255                   260                   265  
           Thr-Gly-Lys-Glu-Met-Phe-Thr-Val-Ala-Glu-Tyr-Trp-Gln-Asn-Asp-  
  
 45  
  
 50  
  
 55

270 275 280  
 Leu-Gly-Ala-Leu-Glu-Asn-Tyr-Leu-Asn-Lys-Thr-Asn-Phe-Asn-His-  
 285 290 285  
 5 Ser-Val-Phe-Asp-Val-Pro-Leu-His-Tyr-Gln-Phe-His-Ala-Ala-Ser-  
 300 305 310  
 Thr-Gln-Gly-Gly-Gly-Tyr-Asp-Met-Arg-Lys-Leu-Leu-Asn-Ser-Thr-  
 10 315 320 325  
 Val-Val-Ser-Lys-His-Pro-Leu-Lys-Ala-Val-Thr-Phe-Val-Asp-Asn-  
 330 335 340  
 His-Asp-Thr-Gln-Pro-Gly-Gln-Ser-Leu-Glu-Ser-Thr-Val-Gln-Thr-  
 15 345 350 355  
 Trp-Phe-Lys-Pro-Leu-Ala-Tyr-Ala-Phe-Ile-Leu-Thr-Arg-Glu-Ser-  
 360 365 370  
 20 Gly-Tyr-Pro-Gln-Val-Phe-Tyr-Gly-Asp-Met-Tyr-Gly-Thr-Lys-Gly-  
 375 380 385  
 Asp-Ser-Gln-Arg-Glu-Ile-Pro-Ala-Leu-Lys-His-Lys-Ile-Glu-Pro-  
 390 395 400  
 25 Ile-Leu-Lys-Ala-Arg-Lys-Gln-Tyr-Ala-Tyr-Gly-Ala-Gln-His-Asp-  
 405 410 415  
 Tyr-Phe-Asp-His-His-Asp-Ile-Val-Gly-Trp-Thr-Arg-Glu-Gly-Asp-  
 420 425 430  
 30 Ser-Ser-Val-Ala-Asn-Ser-Gly-Leu-Ala-Ala-Leu-Ile-Thr-Asp-Gly-  
 435 440 445  
 Pro-Gly-Gly-Ala-Lys-Arg-Met-Tyr-Val-Gly-Arg-Gln-Asn-Ala-Gly-  
 35 450 455 460  
 Glu-Thr-Trp-His-Asp-Ile-Thr-Gly-Asn-Arg-Ser-Glu-Pro-Val-Val-  
 465 470 475  
 Ile-Asn-Ser-Glu-Gly-Trp-Gly-Glu-Phe-His-Val-Asn-Gly-Gly-Ser-  
 40 480 483  
 Val-Ser-Ile-Tyr-Val-Gln-Arg.

#### Revendications

Revendications pour les Etats contractants suivants : BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

- 50 1. Alpha-amylase chimère, qui est thermostable et manifeste un effet négatif réduit sur l'utilisation de la glucoamylase de *A. niger* et de la pullulanase de *B. acidopullulyticus* pour la saccharification de l'amidon, ayant la formule générale I

(I)

55

Q-R-L

dans laquelle Q comprend une partie N-terminale de 55 à 60 résidus d'acides aminés qui a une

homologie d'au moins 75 % avec les 55 résidus d'acides aminés N-terminaux de l'alpha-amylase de *Bacillus amyloliquefaciens* telle que décrite dans Takkinen et coll., J. Biol. Chem. **258** (1983) 1007-1013;

R comprend une partie de formule générale la

(Ia)

58                  60                                      70

Pro-Tyr-Asp-Leu-Tyr-Asp-Leu-Gly-Glu-Phe-X<sub>8</sub>-Gln-Lys-

80

Gly-Thr-Val-Arg-Thr-Lys-Tyr-Gly-Thr-Lys-X<sub>9</sub>-Glu-Leu

88

Gln-X<sub>10</sub>-Ala-Ile-Lys

dans laquelle

X<sub>8</sub> comprend His ou Gln,  
X<sub>9</sub> comprend Gly ou Ser,  
X<sub>10</sub> comprend Ser ou Asp; et

L comprend une partie C-terminale de 390 à 400 résidus d'acides aminés qui a une homologie d'au moins 75 % avec les 395 résidus d'acides aminés C-terminaux de l'alpha-amylase de *Bacillus licheniformis* 584 (ATCC 27811).

**2. Alpha-amylase chimère selon la revendication 1, dans laquelle**

X<sub>8</sub> comprend His,  
X<sub>9</sub> comprend Gly, et  
X<sub>10</sub> comprend Sér.

**3. Alpha-amylase chimère selon la revendication 1, dans laquelle**

X<sub>8</sub> comprend Gln,  
X<sub>9</sub> comprend Ser, et  
X<sub>10</sub> comprend Asp.

**4. Alpha-amylase chimère selon la revendication 1, dans laquelle les homologies sont d'au moins 80 %.**

5. Alpha-amylase chimère selon la revendication 1, dans laquelle les homologies sont d'au moins 90 %.

6. Alpha-amylase chimère selon la revendication 1, dans laquelle Q comprend une partie N-terminale de formule générale Ib

( Ib )

5                      10                      15  
**X<sub>1</sub>-Asn-Gly-Thr-Leu-Met-Gln-Tyr-Phe-Glu-Trp-Tyr-X<sub>2</sub>-Pro-Asn-**  
       20                      25                      30                      35  
**Asp-Gly-Gln-His-Trp-Lys-Arg-Leu-Gln-Asn-Asp-X<sub>3</sub>-Leu-X<sub>4</sub>-Gly-**  
           40                      45                      50  
**Ile-Thr-Ala-Val-Trp-Ile-Pr -Pr -Ala-Tyr-Lys-Gly-X<sub>5</sub>-Ser-Gln-**  
                 55  
**X<sub>6</sub>-Asp-X<sub>7</sub>-Gly-Tyr-Gly:**

dans laquelle

- X<sub>1</sub> comprend Ala-Asn-Leu ou Val,
- X<sub>2</sub> comprend Met ou Thr,
- X<sub>3</sub> comprend Ser-Ala-Tyr ou Ala-Glu-His,
- 5 X<sub>4</sub> comprend Ala-Gly-His ou Ser-Asp-Ile,
- X<sub>5</sub> comprend Thr ou Leu,
- X<sub>6</sub> comprend Ala ou Ser, et
- X<sub>7</sub> comprend Val ou Asn.

10 7. Alpha-amylase chimère selon la revendication 6, dans laquelle

- X<sub>1</sub> comprend Val,
- X<sub>2</sub> comprend Thr,
- X<sub>3</sub> comprend Ala-Glu-His,
- X<sub>4</sub> comprend Ser-Asp-Ile,
- 15 X<sub>5</sub> comprend Leu,
- X<sub>6</sub> comprend Ser, et
- X<sub>7</sub> comprend Asn.

20 8. Alpha-amylase selon la revendication 4, 5, 6 ou 7, dans laquelle L comprend une partie C-terminale de formule générale Ic

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40

45

50

55

(Ic)

5                   90                               95                               100  
           Ser-Leu-His-Ser-Arg-Asp-Ile-Asn-Val-Tyr-Gly-Asp-Val  
                   105                               110                               115  
 10 Val-Ile-Asn-His-Lys-Gly-Gly-Ala-Asp-Ala-Thr-Glu-Asp-Val-Thr-  
                   120                               125                               130  
           Ala-Val-Glu-Val-Asp-Pro-Ala-Asp-Arg-Asn-Arg-Val-Ile-Ser-Gly-  
 15                   135                               140                               145  
           Glu-His-Arg-Ile-Lys-Ala-Trp-Thr-His-Phe-His-Phe-Pro-Gly-Arg-  
                   150                               155                               160  
           Gly-Ser-Thr-Tyr-Ser-Asp-Phe-Lys-Trp-His-Trp-Tyr-His-Phe-Asp-  
 20                   165                               170                               175  
           Gly-Thr-Asp-Trp-Asp-Glu-Ser-Arg-Lys-Leu-Asn-Arg-Ile-Tyr-Lys-  
                   180                               185                               190  
 25 Phe-Gln-Gly-Lys-Ala-Trp-Asp-Trp-Glu-Val-Ser-Asn-Glu-Asn-Gly-  
                   195                               200                               205  
           Asn-Tyr-Asp-Tyr-Leu-Met-Tyr-Ala-Asp-Ile-Asp-Tyr-Asp-His-Pro-  
 30                   210                               215                               220  
           Asp-Val-Ala-Ala-Glu-Ile-Lys-Arg-Trp-Gly-Thr-Trp-Tyr-Ala-Asn-  
                   225                               230                               235  
 35 Glu-Leu-Gln-Leu-Asp-Gly-Phe-Arg-Leu-Asp-Ala-Val-Lys-His-Ile-  
                   240                               245                               250  
           Lys-Phe-Ser-Phe-Leu-Arg-Asp-Trp-Val-Asn-His-Val-Arg-Glu-Lys-  
                   255                               260                               265  
 40 Thr-Gly-Lys-Glu-Met-Phe-Thr-Val-Ala-Glu-Tyr-Trp-Gln-Asn-Asp-  
                   270                               275                               280  
           Leu-Gly-Ala-Leu-Glu-Asn-Tyr-Leu-Asn-Lys-Thr-Asn-Phe-Asn-His-  
 45                   285                               290                               285  
           Ser-Val-Phe-Asp-Val-Pro-Leu-His-Tyr-Gln-Phe-His-Ala-Ala-Ser-  
                   300                               305                               310  
 50 Thr-Gln-Gly-Gly-Gly-Tyr-Asp-Met-Arg-Lys-Leu-Leu-Asn-Ser-Thr-  
 55

315 320 325  
 Val-Val-Ser-Lys-His-Pro-Leu-Lys-Ala-Val-Thr-Phe-Val-Asp-Asn-  
 5 His-Asp-Thr-Gln-Pro-Gly-Gln-Ser-Leu-Glu-Ser-Thr-Val-Gln-Thr-  
 330 335 340  
 Trp-Phe-Lys-Pro-Leu-Ala-Tyr-Ala-Phe-Ile-Leu-Thr-Arg-Glu-Ser-  
 10 345 350 355  
 Gly-Tyr-Pro-Gln-Val-Phe-Tyr-Gly-Asp-Met-Tyr-Gly-Thr-Lys-Gly-  
 360 365 370  
 Asp-Ser-Gln-Arg-Glu-Ile-Pro-Ala-Leu-Lys-His-Lys-Ile-Glu-Pro-  
 15 375 380 385  
 Ile-Leu-Lys-Ala-Arg-Lys-Gln-Tyr-Ala-Tyr-Gly-Ala-Gln-His-Asp-  
 390 395 400  
 Tyr-Phe-Asp-His-His-Asp-Ile-Val-Gly-Trp-Thr-Arg-Glu-Gly-Asp-  
 20 405 410 415  
 Ser-Ser-Val-Ala-Asn-Ser-Gly-Leu-Ala-Ala-Leu-Ile-Thr-Asp-Gly-  
 420 425 430  
 Pro-Gly-Gly-Ala-Lys-Arg-Met-Tyr-Val-Gly-Arg-Gln-Asn-Ala-Gly-  
 25 435 440 445  
 Glu-Thr-Trp-His-Asp-Ile-Thr-Gly-Asn-Arg-Ser-Glu-Pro-Val-Val-  
 30 450 455 460  
 Ile-Asn-Ser-Glu-Gly-Trp-Gly-Glu-Phe-His-Val-Asn-Gly-Gly-Ser-  
 465 470 475  
 Val-Ser-Ile-Tyr-Val-Gln-Arg.  
 35 480 483

- 40 9. Procédé de production d'une alpha-amylase chimère dans lequel:
- (a) on effectue la recombinaison in vivo de la région codante N-terminale du gène de l'alpha-amylase de B. amyloliquefaciens avec la région codante C-terminale du gène de l'alpha-amylase de B. licheniformis pour former des recombinés;
  - 45 (b) on sélectionne les recombinés qui produisent une alpha-amylase chimère qui est thermostable et manifeste un effet négatif réduit sur l'utilisation de la glucoamylase d'A. niger et de la pullulanase de B. acidopullulyticus pour la saccharification de l'amidon;
  - (c) on cultive les recombinés sélectionnés dans un milieu de croissance approprié, et
  - (d) on récupère l'alpha-amylase chimère à partir de la culture.
- 50 10. Procédé de conversion d'amidon en sirop à haute teneur en dextrose, dans lequel:
- (a) on fait réagir de l'amidon avec l'alpha-amylase chimère de la revendication 1, 2, 3, 4, 5, 6 ou 7 pour former des oligosaccharides; et
  - (b) on fait réagir les oligosaccharides formés dans l'étape (a) avec une glucoamylase pour former du dextrose.
- 55 11. Procédé de conversion d'amidon en sirop à haute teneur en dextrose, selon lequel:
- (a) on fait réagir de l'amidon avec l'alpha-amylase chimère de la revendication 8 pour former des oligosaccharides; et

(b) on fait réagir les oligosaccharides formés dans l'étape (a) avec une glucoamylase pour former du dextrose.

**Revendications pour les Etats contractants suivants : AT, ES, GR**

1. Procédé pour la production d'une alpha-amylase chimère comprenant :
  - (a) la recombinaison in vivo de la région codante N-terminale du gène de l'alpha-amylase de B. amyloliquefaciens avec la région codante C-terminale du gène de l'alpha-amylase de B. licheniformis pour former des recombinés ;
  - (b) la sélection des recombinés qui produisent une alpha-amylase chimère qui est thermostable et manifeste un effet négatif réduit sur l'utilisation de la glucoamylase d'A. niger et de la pullulanase de B. acidopullulyticus pour la saccharification de l'amidon ;
  - (c) la mise en culture des recombinés sélectionnés dans un milieu de croissance approprié, et
  - (d) la récupération de l'alpha-amylase chimère de la culture.
2. Procédé pour la conversion d'amidon en sirop à forte teneur en dextrose, comprenant :
  - (a) la mise en réaction de l'amidon avec une alpha-amylase chimère qui est thermostable et manifeste un effet négatif réduit sur l'utilisation de la glucoamylase d'A. niger et de la pullulanase de B. acidopullulyticus ayant la formule générale I

(I)

**Q-R-L**

dans laquelle

Q comprend une partie N-terminale de 55 à 60 résidus d'acides aminés qui a une homologie d'au moins 75 % avec les 55 résidus d'acides aminés N-terminaux de l'α-amylase de *Bacillus amyloliquefaciens* telle que décrite dans Takkinen et al., J. Biol. Chem. 258 (1983) 1007-1013 ;

R comprend une partie de formule générale la

(18)

Pro-Tyr-Asp-Leu-Tyr-Asp-Leu-Gly-Glu-Phe-X<sub>8</sub>-Gln-Lys-

Gly-Thr-Val-Arg-Thr-Lys-Tyr-Gly-Thr-Lys-X<sub>9</sub>-Glu-Leu

Gln-X<sub>10</sub>-Ala-Ile-Lys

dans laquelle

X<sub>8</sub> comprend His ou Gln,

X<sub>9</sub> comprend Gly ou Ser,

$X_{10}$  comprend Ser ou Asp ; et

L comprend une partie C-terminale de 390 à 400 résidus d'acides aminés qui a une homologie d'au moins 75 % avec les 395 résidus d'acides aminés C-terminaux de l'alpha-amylase de Bacillus licheniformis 584 (ATCC 27811), pour former des oligosaccharides ; et

(b) on met en réaction les oligosaccharides formés dans l'étape (a) avec une glucoamylase pour former du dextrose.

3. Procédé selon la revendication 2, dans lequel dans l'alpha-amylase chimère,
- X<sub>8</sub> comprend His,
- X<sub>9</sub> comprend Gly, et
- X<sub>10</sub> comprend Ser.





(Ic)

5                   90                   95                   100  
           Ser-Leu-His-Ser-Arg-Asp-Ile-Asn-Val-Tyr-Gly-Asp-Val  
                   105                   110                   115  
 10 Val-Ile-Asn-His-Lys-Gly-Gly-Ala-Asp-Ala-Thr-Glu-Asp-Val-Thr-  
                   120                   125                   130  
       Ala-Val-Glu-Val-Asp-Pro-Ala-Asp-Arg-Asn-Arg-Val-Ile-Ser-Gly-  
 15                   135                   140                   145  
       Glu-His-Arg-Ile-Lys-Ala-Trp-Thr-His-Phe-His-Phe-Pro-Gly-Arg-  
                   150                   155                   160  
       Gly-Ser-Thr-Tyr-Ser-Asp-Phe-Lys-Trp-His-Trp-Tyr-His-Phe-Asp-  
 20                   165                   170                   175  
       Gly-Thr-Asp-Trp-Asp-Glu-Ser-Arg-Lys-Leu-Asn-Arg-Ile-Tyr-Lys-  
                   180                   185                   190  
 25 Phe-Gln-Gly-Lys-Ala-Trp-Asp-Trp-Glu-Val-Ser-Asn-Glu-Asn-Gly-  
                   195                   200                   205  
       Asn-Tyr-Asp-Tyr-Leu-Met-Tyr-Ala-Asp-Ile-Asp-Tyr-Asp-His-Pro-  
 30                   210                   215                   220  
       Asp-Val-Ala-Ala-Glu-Ile-Lys-Arg-Trp-Gly-Thr-Trp-Tyr-Ala-Asn-  
                   225                   230                   235  
       Glu-Leu-Gln-Leu-Asp-Gly-Phe-Arg-Leu-Asp-Ala-Val-Lys-His-Ile-  
 35                   240                   245                   250  
       Lys-Phe-Ser-Phe-Leu-Arg-Asp-Trp-Val-Asn-His-Val-Arg-Glu-Lys-  
                   255                   260                   265  
 40 Thr-Gly-Lys-Glu-Met-Phe-Thr-Val-Ala-Glu-Tyr-Trp-Gln-Asn-Asp-  
                   270                   275                   280  
       Leu-Gly-Ala-Leu-Glu-Asn-Tyr-Leu-Asn-Lys-Thr-Asn-Phe-Asn-His-  
 45                   285                   290                   285  
       Ser-Val-Phe-Asp-Val-Pro-Leu-His-Tyr-Gln-Phe-His-Ala-Ala-Ser-

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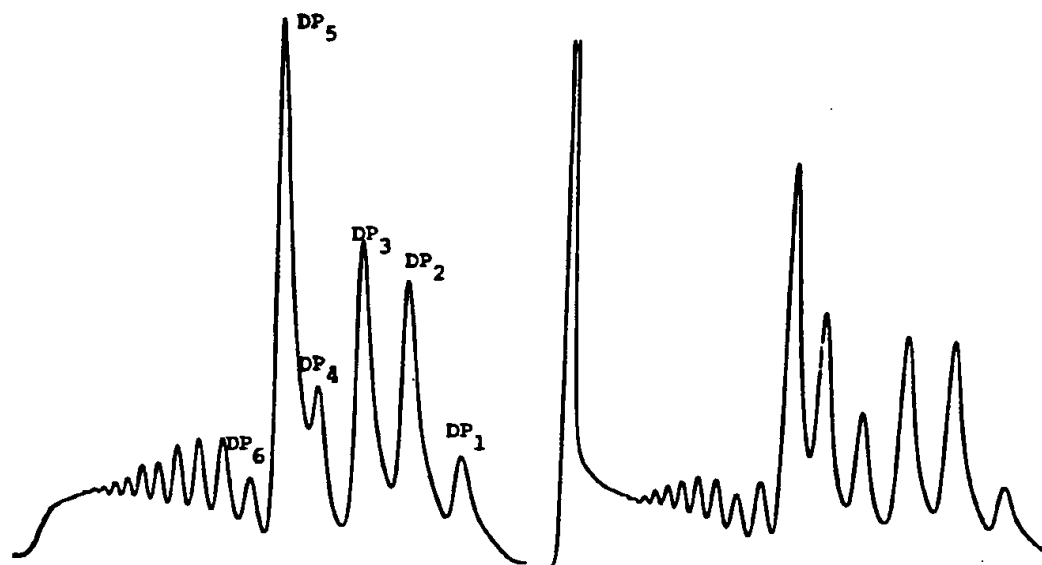
300 305 310  
 Thr-Gln-Gly-Gly-Gly-Tyr-Asp-Met-Arg-Lys-Leu-Leu-Asn-Ser-Thr  
 5 315 320 325  
 Val-Val-Ser-Lys-His-Pro-Leu-Lys-Ala-Val-Thr-Phe-Val-Asp-Asn-  
 330 335 340  
 His-Asp-Thr-Gln-Pro-Gly-Gln-Ser-Leu-Glu-Ser-Thr-Val-Gln-Thr-  
 10 345 350 355  
 Trp-Phe-Lys-Pro-Leu-Ala-Tyr-Ala-Phe-Ile-Leu-Thr-Arg-Glu-Ser-  
 360 365 370  
 15 Gly-Tyr-Pro-Gln-Val-Phe-Tyr-Gly-Asp-Met-Tyr-Gly-Thr-Lys-Gly-  
 375 380 385  
 Asp-Ser-Gln-Arg-Glu-Ile-Pro-Ala-Leu-Lys-His-Lys-Ile-Glu-Pro-  
 20 390 395 400  
 Ile-Leu-Lys-Ala-Arg-Lys-Gln-Tyr-Ala-Tyr-Gly-Ala-Gln-His-Asp-  
 405 410 415  
 Tyr-Phe-Asp-His-His-Asp-Ile-Val-Gly-Trp-Thr-Arg-Glu-Gly-Asp-  
 25 420 425 430  
 Ser-Ser-Val-Ala-Asn-Ser-Gly-Leu-Ala-Ala-Leu-Ile-Thr-Asp-Gly-  
 435 440 445  
 30 Pro-Gly-Gly-Ala-Lys-Arg-Met-Tyr-Val-Gly-Arg-Gln-Asn-Ala-Gly-  
 450 455 460  
 Glu-Thr-Trp-His-Asp-Ile-Thr-Gly-Asn-Arg-Ser-Glu-Pro-Val-Val-  
 465 470 475  
 35 Ile-Asn-Ser-Glu-Gly-Trp-Gly-Glu-Phe-His-Val-Asn-Gly-Gly-Ser-  
 480 483  
 Val-Ser-Ile-Tyr-Val-Gln-Arg.

40

45

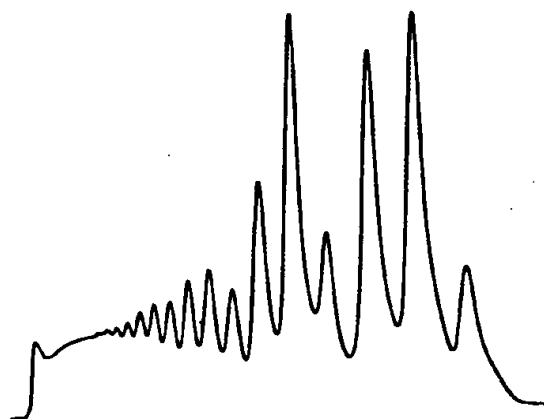
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B. LICHENIFORMIS

B. AMYLOLIQUEFACIENS



QL1864

FIG. 1

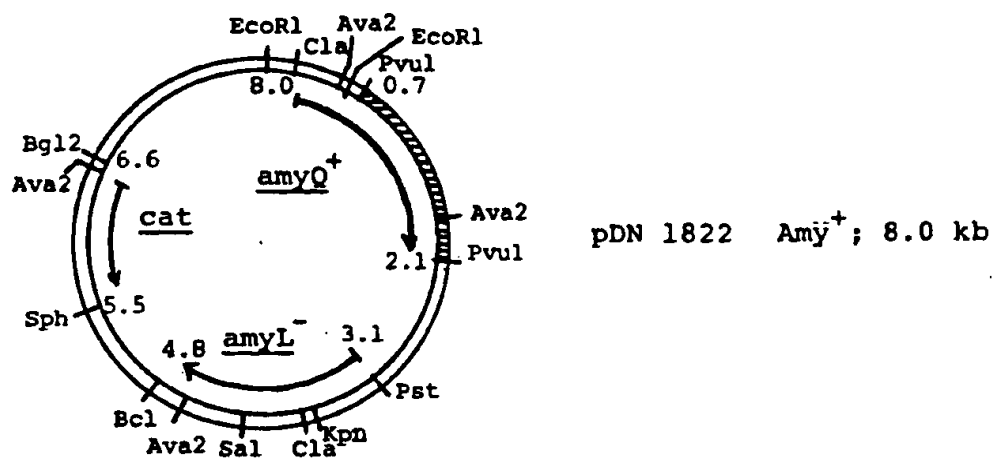


FIG. 2

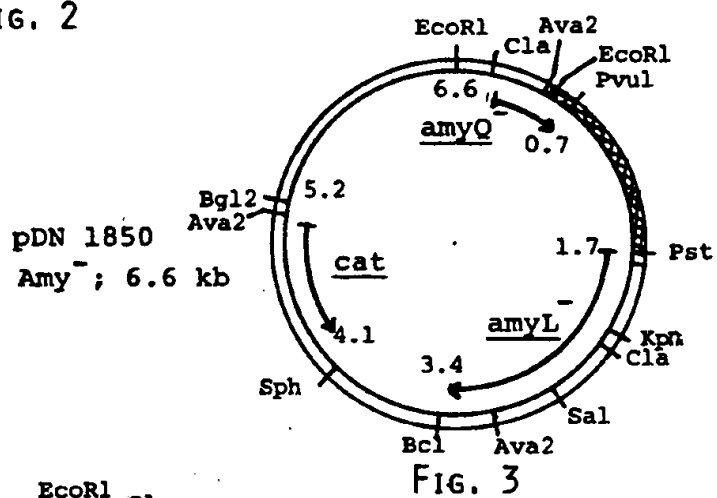


FIG. 3

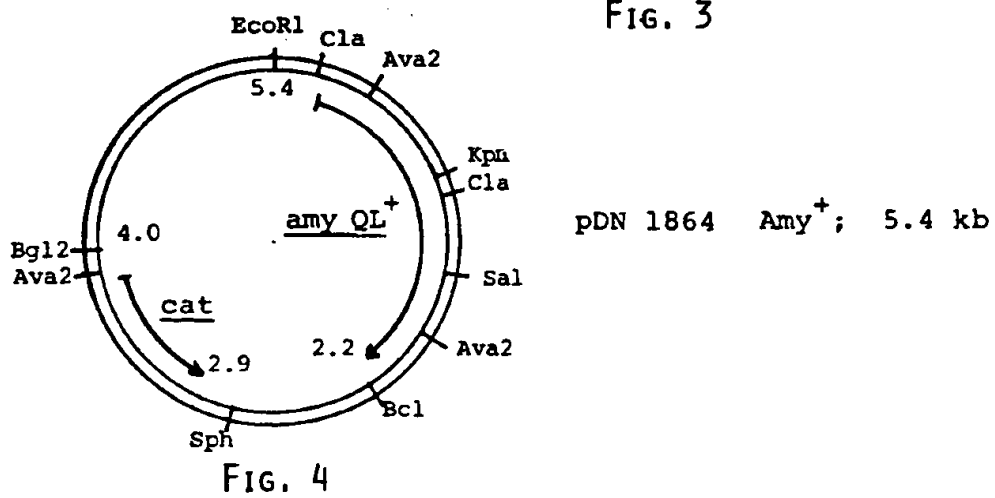


FIG. 4